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STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF MODENESE COW MILK IN PARMIGIANO-REGGIANO CHEESE PRODUCTION [1]

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Introduction

Parmigiano-Reggiano cheese production consists in the formation and dehydration of a acid-rennet curd. The basic requirement of milk is its specific rennet-coagulation aptitude (1). The milk must have: a medium high casein content, caseins of a genetically favourable type, a good colloidal calcium phosphate content, a correct degree of titratable acidity, a moderate content of somatic cells and an optimal aptitude to rennet-coagulation (good reactivity with rennet, high degree of curd firming capacity and good ability to contraction and expulsion of whey) (2).

All these properties are influenced, more or less, by several factors. Genetic factors, in particular, play a very important role in the production of Parmigiano-Reggiano cheese (3-5). One of the cattle breeds reared in the Parmigiano-Reggiano production area is Modenese, also named Bianca Val Padana. Its population size today is about 500 (204 cows) head registered in the Herdbook, bred in the provinces of Modena, Mantova and Reggio Emilia (Northern Italy). According to FAO (6), Modenese is considered a endangered breed.

Modenese cattle has a white coat and black hooves, a black muzzle with a characteristic depigmented inverted V, white horns black tipped; 800-900 kg live weight for males and 650 kg for females; 155-160 cm withers height males and 145-150 cm females. This breed seems to originate from crosses of local Jurassic cattle with Podolic animals in the period after the fall of the Roman Empire. Baker and Manwell (7) include it among the Podolic Italian breeds derived from the *primigenius*, although recognising the initial contribution of *Bos brachyceros*. On the other hand, the phylogenetic analysis made by Astolfi *et al.* (8) seems to give preference to the origin from the *brachyceros* branch since it is included among the lowland cattle populations living south of the Alpes rather than among the Podolic ones. The decay of Modenese cattle (in origin three purpose breed) arises from the introduction of the most productive Friesian cattle.

The aim of this note was to report data related to the structural and functional characteristics of Modenese (MO) cow milk in the Parmigiano-Reggiano cheese pro-

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duction, and compare them to those of Italian Friesian milk (MO *vs* IF).

Casein content and casein number

Casein is the true raw material of cheese. On casein most of the rheological characteristics of the curd, the contracting capacity of cheese mass and the yield of dairy transformation depend, as well as the physico-chemical and functional properties of the finished product. Milks with low casein content originate weak curds, characterised by low elasticity, from which excessively moist cheese masses with scarce contractility arise (9, 10) and therefore cheese with structural and organoleptic alterations. The cheese yield, which is strongly related to the casein content, results lower (11).

Relevant differences in protein and casein content and casein number emerge from surveys carried out on herd milk samples belonging to the principal cattle breeds reared in Parmigiano-Reggiano production area (12) (Table 1).

The Modenese cow milk contains 0.4 percent more units of crude protein (3.48 *vs* 3.01 g/100g) and casein (2.75 *vs* 2.32 g/100g). This difference has a great economic relevance considering that to each part of casein about three parts of ripened cheese correspond. Casein number is clearly higher in Modenese milk (79.05 *vs* 76.92%). The Italian Friesian cow milk shows a casein number similar to the conventional value (77%), while the Modenese milk is 2 percent units higher. The values concerning Modenese milk result in accordance to other researches (13).

Distribution of casein fractions

Distribution of casein fractions (α_{s1}, α_{s2}, β and k), even if rather constant, could undergo such variation that the micellar dispersion degree can be affected significantly and consequently the properties of the whole casein system can vary, with great influence on the rennet-coagulation characteristics. The system is particularly influenced by the proportional variations of k-casein, the bearing element of the micellar structure.

Table 1 - Protein and casein contents and casein number (12).
Tabella 1 - Contenuti di proteina e caseina e indice di caseina (12).

		Modenese[(1)] *Modenese[(1)]*	It. Friesian[(1)] *Frisona it.[(1)]*	P
Protein (Nx6.38) *Proteina*	g/100g	3.48 ± 0.14	3.01 ± 0.11	**
Casein *Caseina*	g/100g	2.75 ± 0.11	2.32 ± 0.10	**
Casein number *Indice di caseina*	–	79.05 ± 1.79	76.92 ± 1.70	*

(1) 12 herd milk samples; *12 campioni di latte di allevamento*
* P≤0.05; ** P≤0.01

The percentage repartition of caseins of Modenese cow differ to that of Italian Friesian cow (12) (Table 2), especially concerning the k-casein and α_{s2}-casein. The casein of Modenese cow contains more k-casein (12.28%) than that of Italian Friesian (11.25%). Such a difference is probably correlated with the higher expression level of the B allele toward the A allele (14-17), the former with higher frequency in Modenese cow.

Table 2 - Percentage distribution of the caseins (12).
Tabella 2 - Ripartizione percentuale delle caseine (12).

	Modenese[1] *Modenese[1]*	It. Friesian[1] *Frisona it.[1]*	P
k_p major k-casein [2] *k_p k-caseina principale [2]*	6.77 ± 0.61	5.46 ± 0.34	**
k_m "minor" k-caseins [2] *k_m k-caseine "minori" [2]*	5.51 ± 1.07	5.79 ± 0.70	ns
α_{s2}-casein *α_{s2}-caseina*	13.61 ± 0.80	11.39 ± 0.49	**
α_{s1}-casein *α_{s1}-caseina*	34.15 ± 1.61	36.27 ± 0.92	*
$\beta(+\gamma)$-casein *$\beta(+\gamma)$-caseina*	37.42 ± 1.73	38.09 ± 0.95	ns
γ_m other γ-caseins *γ_m altre γ-caseine*	2.54 ± 1.04	3.00 ± 1.18	ns
k-casein ($k_p + k_m$) *k-caseina ($k_p + k_m$)*	12.28 ± 1.36	11.25 ± 0.67	*
α_s-casein ($\alpha_{s1} + \alpha_{s2}$) *$\alpha_s$-caseina ($\alpha_{s1} + \alpha_{s2}$)*	47.76 ± 1.44	47.66 ± 0.87	ns
$(\beta + \gamma)$-casein *$(\beta + \gamma)$-caseina*	39.96 ± 1.27	41.09 ± 0.95	*
k to α_{s1} *ratio k/α_{s1}*	0.36 ± 0.05	0.31 ± 0.02	*

[1] 12 herd milk samples (RP-HPLC according to Visser *et al.* (25)); *12 campioni di latte di allevamento (RP-HPLC secondo Visser* et al. *(25)).*

[2] Major k-casein (k_p): is the principal fraction of the k-casein, non-glycosylated, corresponding to the chromatographic peak which identifies the genetic variant(s) of the protein; the other fractions of the k-casein are grouped in k_m and named "minor" k-caseins; *k-caseina principale (k_p): è la frazione principale della k-caseina, non-glicosilata, corrispondente al picco cromatografico che identifica la variante(i) genetica della proteina; le altre frazioni della k-caseina sono raggruppate in k_m e chiamate k-caseine "minori".*

ns, P>0.05; * P≤0.05; ** P≤0.01

The different distribution of k-casein is mainly related to its principal fraction (k_p, non glycosylated), which proportion in Modenese (6.77%) is higher than in Italian Friesian (5.46%). The Modenese is also characterised by a different *ratio* between glycosylated k-casein (k_m) and non glycosylated k-casein (k_p) (0.81 *vs* 1.06).

Anyway, the observation is of undoubted interest (18, 19), especially with reference to the influence of k-casein proportional variations on the size (20) and the reactivity toward chymosin (21-24) of micelles (more k-casein, smaller micelles, more reactive micelles).

The difference regarding α_{s2}-casein is as much important. The casein of Modenese milk is richer in α_{s2}-casein (13.61%) than that of Italian Friesian milk (11.39%).

The casein of Modenese milk contains lower amount of α_{s1}-casein; this latter characteristic further differentiate the micellar system of Modenese, which κ/α_{s1} *ratio*, equal to 0.36, is significantly higher than that of the Italian Friesian (0.31) (Table 2).

Calcium, phosphorus and chloride

The Modenese cow milk is richer of calcium (122.1 *vs* 116.9 mg/100ml) and phosphorus (101.9 *vs* 91.7 mg/100ml) than the Italian Friesian cow milk (26) (Table 3).

Since about 2/3 of calcium and a half of the phosphorus are integral part of the micellar system, such differences appear to be related principally to the different casein content of the two milks. Modenese milk, because of its remarkable richness of phosphorus, tends to be characterised on average by a lower Ca/P ratio (1.20 *vs* 1.27).

Also the chloride content (27) (Table 3) varies considerably: the Modenese milk has a chloride ion concentration markedly lower (82.1 *vs* 101.4 mg/100g) and therefore more favourable for cheesemaking.

Micellar system composition

The colloidal fraction of the milk, structured in micelles, is the basic constituent of the cheese mass. Colloidal calcium phosphate, strictly associated to caseins, is essential for the formation and the integrity of this particular structure; furthermore, it plays a very important role in all phases of milk coagulation. Compared to that of Italian Friesian, Modenese milk (26) (Table 4) is characterised by a higher concentration of all principal constituents of the micellar system; it contains more casein and there are also relevant differences regarding colloidal phosphorus (51.7 *vs* 48.0 mg/100ml), particularly in relation to the fraction covalently bound to the casein (24.8 *vs* 22.4 mg/100ml). Moreover, from these surveys can be observed that the micellar system of Modenese milk shows generally a lower mineralisation degree compared to that of Italian Friesian (26). In fact, it contains a lower content of colloidal inorganic phosphate per 100 casein units (2.97 *vs* 3.07%).

Table 3 - Calcium, phosphorus and chloride contents (mg/100ml) (26).
Tabella 3 - Contenuti di calcio, fosforo e cloruri (mg/100ml) (26).

	Modenese[1] *Modenese[1]*	It. Friesian[1] *Frisona it.[1]*
Calcium, Ca *Calcio, Ca*	122.1	116.9
Colloidal Ca *Ca colloidale*	82.2 ± 3.5	78.5 ± 4.1
Soluble Ca *Ca solubile*	39.9 ± 2.2	38.4 ± 1.4
Phosphorus, P *Fosforo, P*	101.9	91.7
Colloidal P *P colloidale*	51.7 ± 1.7	48.0 ± 2.3
Soluble P *P solubile*	48.8 ± 2.5	42.3 ± 2.6
Ca to P *ratio Ca/P*	1.20	1.27
Chloride [2] *Cloruri[2]*	82.1 ± 14.9	101.4 ± 10.0

[1] 10 dairy herds (4 milk samples for each herd)*; 10 allevamenti (4 campioni di latte per allevamento)*

[2] Data from Mariani *et al.* (27): 12 herd milk samples for each breed (mg Cl^-/100g); *Dati ripresi da Mariani* et al. *(27): 12 campioni di latte di allevamento per ciascuna razza (mg Cl^-/100g)*

Genetic variants of caseins and of β-lactoglobulin

The polymorphism of α_{s1}-, β- and k- caseins and of β-lactoglobulin are a very particular aspect in the complex subject of milk quality. The genetic structure of Modenese (28) is markedly different than that of Italian Friesian (29) concerning the casein variants (Table 5).

In Modenese milk there is a substantial equilibrium between the frequencies of the A and B k-casein variants (0.505A *vs* 0.495B), while in Italian Friesian the A variant is remarkably more frequent than B (0.755A *vs* 0.245B). The same happens for the two variants more representative at the β-casein *locus* (A and B).

The influence of the β-lactoglobulin genetic types is remarkable. Cows with β-lactoglobulin A type give a milk with higher content of whey proteins, while cows with β-lactoglobulin B type produce a milk on average more rich of casein, with significant effects particularly on cheese yield. Modenese milk is characterised by a higher frequency of β-lactoglobulin B variant (0.722 MO *vs* 0.541 IF) than that of Italian Friesian (30).

Table 4 - Micellar system composition (mg / 100 ml milk) (26).
Tabella 4 - Composizione del sistema micellare (mg / 100 ml di latte) (26).

	Modenese[1] *Modenese[1]*	It. Friesian[1] *Frisona it.[1]*
Casein *Caseina*	2780 ± 10	2560 ± 11
Colloidal calcium *Calcio colloidale*	82.2 ± 3.5	78.5 ± 4.1
Colloidal phosphorus *Fosforo colloidale*	51.7 ± 1.7	48.0 ± 2.3
Casein P *P caseina*	24.8 ± 1.4	22.4 ± 0.8
Coll. inorg. P *P coll. inorg.*	26.9 ± 2.0	25.6 ± 1.8
Colloidal magnesium *Magnesio colloidale*	3.5 ± 0.3	3.5 ± 0.3
Colloidal citric acid *Acido citrico colloidale*	9.9 ± 2.3	8.7 ± 1.8

[1] 10 dairy herds (4 milk samples for each herd); *10 allevamenti (4 campioni di latte per allevamento)*

Table 5 - Genetic variants of caseins and β-lactoglobulin.
Tabella 5 - Varianti genetiche delle caseine e della β-lattoglobulina.

	Alleles *Alleli*	Modenese[1] *Modenese[1]*	It. Friesian[2] *Frisona it.[2]*
α_{s1}-Cn	B	0.820	0.969
	C	0.180	0.031
β-Cn	A	0.797	0.952
	B	0.156	0.046
	C	0.047	<0.02
κ-Cn	A	0.505	0.755
	B	0.495	0.247
β-Lg	A	0.277	0.459
	B	0.722	0.541
	D	0.001	

[1] 424 individual milk samples collected from 59 dairy herds (28)*; 424 campioni di latte individuale raccolti da 59 allevamenti (28)*

[2] 748 individual milk samples collected from 60 dairy herds (29)*; 748 campioni di latte individuale raccolti da 60 allevamenti (29)*

Micelle size

The genetic differences in the principal casein systems assume a very particular significance from the dairy-technological point of view. Milks characterised by the presence of k-casein B variant have a higher content, absolute and relative, of k-casein. This implies the presence of micelles with a lower average diameter. Concerning the coagulation properties, smaller micelles have higher reactivity with the rennet and a higher rate of curd formation, with positive repercussions on the rheological characteristics of the curd (31-32).

Also the micellar structure of the milks with the β-casein B variant is characterised by micelles with a lower average micellar diameter with respect to A variant, with positive repercussions on the reactivity with rennet and the aggregation rate of the paracaseinate micelles (33-34). These observations are partially confirmed by Morini *et al.* (21) who observed an average micellar diameter lower for Modenese milk (62 *vs* 68 nm) (Table 6).

Table 6 - Micelle size distribution (21).
Tabella 6 - Distribuzione delle micelle (21).

		Modenese[1] *Modenese[1]*	It. Friesian[2] *Frisona it.[2]*
Submicelles (under 12 nm) *Submicelle (fino a 12 nm)*	%	39.82	37.57
Small micelles (12-68 nm) *Micelle piccole (12-68 nm)*	%	40.90	36.58
Medium micelles (68-162 nm) *Micelle medie (68-162 nm)*	%	17.57	24.31
Large micelles (over 162 nm) *Micelle grandi (sopra 162 nm)*	%	1.71	1.54
Mean micellar diameter *Diametro micellare medio*	nm	62	68

(1) 1753 particles measured on microphotographies; *Misurate 1753 particelle su microfotografie*

(2) 3040 particles measured on microphotographies; *Misurate 3040 particelle su microfotografie*

Note: milk samples were examined by TEM (transmission electron microscopy) after freeze-fracturing sample preparation according to Buchheim (35) and Resmini and Volonterio (36).

Nota: i campioni di latte, preparati con la tecnica freeze-fracturing secondo Buchheim (35) e Resmini e Volonterio (36), sono stati esaminati al microscopio elettronico.

Rennet coagulation properties

Acidity markedly influences the rennet-coagulation aptitude of the milk, both in the primary and secondary phase of the coagulation. The fundamental role is played by pH (whose values are negatively related to those of titratable acidity) that strongly influences the primary reaction of the rennet and the curd firming rate.

According to the observations of Pecorari *et al.* (4), Modenese cow milk is characterised by a clearly higher titratable acidity (3.59 *vs* 3.29 °SH/50ml) with respect to that of the Italian Friesian.

Modenese cow milk has very peculiar coagulation properties (Table 7), confirming its several specific characteristics. It coagulates with tendentially longer times (17.9 *vs* 16.5 min), has a reasonable curd firming time (10.5 *vs* 14.1 min) but not such as to let the curd reach technologically favourable values, both in terms of firmness a_{30} (25.4 *vs* 22.2 mm) and in terms of resistance to compression (30.3 *vs* 29.8 g) and resistance to cut (51.0 *vs* 47.9 g); however, the curd reaches values of consistence and strength lightly higher than those of Italian Friesian.

Anyway the rennet-coagulation properties of Modenese milk are not technologically suitable for Parmigiano-Reggiano cheese production, even if better than those of Italian Friesian: probably because of the lower content of colloidal calcium phos-

Table 7 - Rennet-coagulation properties (4).
Tabella 7 - Proprietà di coagulazione presamica (4).

		Modenese[1] *Modenese[1]*	It. Friesian[2] *Frisona it.[2]*
Titratable acidity *Acidità titolabile*	°SH/50ml	3.59	3.29
Clotting time r [3] *Tempo coagulazione r [3]*	min	17.9	16.5
Curd firming time k_{20} [3] *Tempo rassodamento k_{20} [3]*	min	10.5	14.1
Curd firmness a_{30} [3] *Consistenza coagulo a_{30} [3]*	mm	25.4	22.2
Resistance to compression [4] *Resistenza alla compressione [4]*	g	30.3	29.8
Resistance to cut [4] *Resistenza al taglio [4]*	g	51.0	47.9

[1] 75 individual milk samples; *75 campioni di latte individuale*
[2] 239 individual milk samples; *239 campioni di latte individuale*
[3] Lactodynamography according to McMahon and Brown (37); *Lattodinamografia secondo McMahon e Brown (37)*
[4] Gelometry according to Annibaldi (38); *Gelometria secondo Annibaldi (38)*

phate per casein unit (lower mineralisation degree of the micelle), the Modenese milk tends to form a curd of mealy type, less elastic and considerably different from those "gelatinous" typical of the milk of other breeds, as Italian Brown and Reggiana (4, 39).

Conclusions

Modenese milk is rich of casein and is characterised by high casein number. These peculiarities are favourable to a high cheese yield in dairy transformation.

Nevertheless, the Modenese coagulum tends to be "mealy", provided of rheological properties different from those characterising the "gelatinous" coagulum considered technologically more suitable for Parmigiano-Reggiano cheese. Probably, these unfavourable rheological peculiarities of the Modenese milk are due to the lower mineralisation degree of the native micelles.

Key words: cow milk, Modenese cattle, Italian Friesian cattle, Parmigiano-Reggiano cheese
Parole chiave: latte di vacca, Modenese, Frisona Italiana, Parmigiano-Reggiano

SUMMARY - The aim of this study was to compare the characteristics of Modenese cow milk (MO) with those of Italian Friesian (IF). Modenese milk is rich of casein (2.75 *vs* 2.32%) and characterised by a high casein number (79.05 *vs* 76.92%). Casein has high proportions of α_{s2} (13.61 *vs* 11.39%) and k-casein (12.28 *vs* 11.25%); the k to α_{s1} *ratio* is favourable (0.36 *vs* 0.31). About half of the k-casein variants are B type (49 *vs* 25%). Modenese milk is sufficiently provided with total calcium (122.1 *vs* 116.9mg/100ml) and rich of total phosphorus (101.9 *vs* 91.7mg/100ml); it contains less chloride (82.1 *vs* 101.4mgCl^-/100g). Mineralisation degree of micellar system seems not sufficiently adequate (2.97 *vs* 3.07% colloidal inorganic PO_4). Micelles have smaller size (62 *vs* 68nm). Modenese milk has a good titratable acidity (3.59 *vs* 3.29 °SH/50ml). It shows a rather long coagulation time (17.9 *vs* 16.5min); curd firmness (a_{30}) is substantially poor (25.4 *vs* 22.2mm). Although the MO values of curd resistance to compression (30.3 *vs* 29.8g) and to cut (51.0 *vs* 47.9g) are better than IF, anyway they have to be considered low. On a whole, the coagulum tends to be "mealy", provided of rheological properties different from those characterising the "gelatinous" coagulum, considered technologically more suitable for Parmigiano-Reggiano cheese manufacture.

RIASSUNTO - Caratteristiche strutturali e funzionali del latte di vacche di razza Modenese nella produzione del Parmigiano-Reggiano.

Lo scopo di questo studio è stato quello di confrontare le principali caratteristiche strutturali e funzionali del latte di vacche di razza Modenese con quelle di vacche di razza Frisona italiana. Il latte della Modenese risulta più ricco di caseina (2,75 *vs* 2,32%) e caratterizzato da un elevato indice di caseina (79,05 *vs* 76,92%). La caseina è contraddistinta da un'elevata proporzione delle frazioni α_{s2}-caseina (13,61 *vs* 11,39%) e k-caseina (12,28 *vs* 11,25%); il rapporto k/α_{s1} risulta tecnologicamente favorevole alla Modenese (0,36 *vs* 0,31). La frequenza allelica della variante B al

locus k-caseina raggiunge valori prossimi al 50% (49 *vs* 25%). Il latte della Modenese è sufficientemente provvisto di calcio totale (122,1 *vs* 116,9 mg/100ml), particolarmente ricco di fosforo totale (101,9 *vs* 91,7 mg/100ml) e caratterizzato da un minor contenuto di cloruri (82,1 *vs* 101,4 $mgCl^-$/100g). Il grado di mineralizzazione del sistema micellare sembra non essere sufficientemente adeguato (2,97 *vs* 3,07% di fosfato colloidale inorganico). Le micelle hanno un diametro mediamente più piccolo (62 *vs* 68 nm). Il latte della Modenese risulta provvisto di una buona acidità titolabile (3,59 *vs* 3,29 °SH/50ml). Per quanto riguarda le caratteristiche di coagulazione presamica, il latte della Modenese mostra un tempo di coagulazione (r) piuttosto lento (17,9 *vs* 16,5 min) ed il coagulo presenta una consistenza (a_{30}) sostanzialmente bassa (25,4 *vs* 22,3 mm). Anche se i valori di resistenza alla compressione (30,3 *vs* 29,8 g) e di resistenza al taglio (51,0 *vs* 47,9 g) del coagulo sono migliori rispetto a quelli della Frisona, essi devono essere considerati, in assoluto, piuttosto carenti. In generale, il coagulo della Modenese tende ad essere "farinoso", provvisto di proprietà reologiche diverse rispetto a quelle del caratteristico coagulo "gelatinoso", considerato tecnologicamente più idoneo per la produzione di Parmigiano-Reggiano.

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